

CLAIMS

1. An oral vaccine comprising a recombinant lactic acid bacterium capable of expressing a heterologous antigen intracellularly and/or on the surface of the bacterium, wherein the bacterium is *Lactobacillus plantarum* and can elicit an immune response and/or immunogenicity against the heterologous antigen.

2. A vaccine according to claim 1 wherein the recombinant *Lactobacillus plantarum* comprises an expression vector capable of causing expression of the heterologous antigen intracellularly and/or exposure on the cell surface, optionally under conditions present in the gastrointestinal tract.

3. A vaccine according to claim 1 or 2 wherein the heterologous antigen can induce immunogenicity against a pathogenic microorganism, optionally a heterologous antigen specific for a mucosa colonising pathogen or pathogen entering the body via the mucosa, such as via the oral route.

4. A vaccine according to any of the preceding claims wherein the heterologous antigen induces immunogenicity against a pathogenic microorganism colonising the gastrointestinal tract.

5. A vaccine according to any of the preceding claims wherein the pathogenic microorganism is herpes virus, rubella virus, influenza virus, mumps virus, measles virus, poliomyelitis virus, rotavirus, respiratory syncytial virus, *Campylobacter* species, *Chlamydial* organisms, species of the genus *Cryptosporidium*, cytomegalovirus, human immunodeficiency virus, *Actinomyces* species, *Entamoeba histolytica*, arenaviruses, arboviruses, *Clostridium botulinum*, species of the genus *Candida*, *Vibrio cholera*, *Cryptococcus neoformans*, EHEC strains of *E.coli* O157:H7, O26:H11, O111:H8 and O104:H21, ETEC strains of *E.coli*, strains of *E.coli* shown to possess enteroinvasiveness (EIEC), EPEC strains of *E.coli*, EAaggEC strains of *E.coli*., DAEC strains of *E.coli*, filoviridae, parvovirus, *Filarioidea*, *Staphylococcus aureus*, species of the genus *Clostridium perfringens*, *Helicobacter pylori*, Caliciviruses, *Giardia lamblia*, *Neisseria gonorrhoeae*, hantaviruses, hepatitis viruses types A, B, C, D, E, *Legionellae* strains, *Mycobacterium leprae*, *Listeria monocytogenes*, species of the genus *Clostridium perfringens*, *Borrelia burgdorferi*, *Pseudomonas pseudomallei*, Epstein Barr virus,

*Onchocerca volvulus*, Poxvirus, *Bordetella pertussis*, *Yersinia pestis*, *Coxiella burnetti*, rabies virus, *Treponema pallidum*, *Mycobacterium tuberculosis*, *Salmonella typhi*, a (eukaryotic parasite) causing malaria, *pneumocystis pneumonia*, an agent causing toxoplasmosis, or any combination thereof.

5 6. A vaccine according to any preceding claim which elicits a protective response against a rotavirus, respiratory syncytial virus, *Mycobacterium tuberculosis*, human immunodeficiency virus, *E.coli*, *Vibrio cholera*, streptococci and/or chlamydia.

10 7. A vaccine according to any of the preceding claims wherein the heterologous antigen is a viral and/or bacterial antigen optionally a (gp160) envelope protein of the HIV virus, a surface glycoprotein of a *Leishmania* parasite, Shiga-like toxin, *Shigella* lipopolysaccharide antigen, *Escherichia coli* fimbrial antigen, a CFA antigen of an enterotoxigenic *Escherichia coli* strain, anthrax toxin, pertussis toxin, tetanus toxin.

15 8. A vaccine according to any of claims 1-4 wherein the heterologous antigen is a human allergen or the heterologous antigen is specific for tetanus.

9. A vaccine according to any of the preceding claims which can induce protective immunogenicity.

20 10. A vaccine according to any of the preceding claims formulated as a single dose vaccine.

11. A vaccine according to any of the preceding claims wherein the recombinant *Lactobacillus plantarum* expresses the heterologous antigen intracellularly and/or on the cell surface to a degree exceeding that of *Lactobacillus plantarum* 80 expressing  $\beta$ -galactosidase.

25 12. A vaccine according to any of the preceding claims wherein the recombinant *Lactobacillus plantarum* comprises a homologous expression and/or secretion signal, optionally in an expression vector for *Lactobacilli*, preferably for *Lactobacillus plantarum*.

30 13. A vaccine according to any of the preceding claims wherein the recombinant *Lactobacillus plantarum* strain exhibits a persistence (in the individual vaccinated) exceeding 5 days, preferably exceeding 9 days, suitably more than 15 or even 20 days

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14. A vaccine according to any of the preceeding claims wherein the recombinant *Lactobacillus plantarum* exhibits a persistence longer than that of *L. plantarum* 80, preferably longer than that of *L. plantarum* NCIMB 8826, under equivalent conditions.

5      15. A vaccine according to any of the preceeding claims formulated administration to a human, such as an infant, immunocompromised person, elderly person or a normally healthy infant, child or adult.

16. A vaccine according to any of the preceeding claims wherein the recombinant *Lactobacillus plantarum* is a recombinant *Lactobacillus plantarum* 256.

10      17. A vaccine according to any of the preceeding claims wherein the vaccine comprises at least one adjuvant or a pharmacologically acceptable carrier.

18. A recombinant *Lactobacillus plantarum*, optionally a recombinant strain of *Lactobacillus plantarum* 256, as defined in any of the preceeding vaccine claims.

15      19. A bacterium according to claim 18 which is of non-human origin.

20. A non-human and/or non-human foodstuff *Lactobacillus* bacterium which has been modified to express a heterologous antigen and to elicit an immune response in an individual.

21. A bacterium according to claim 20 wherein:

20      (a) the naturally occurring or unmodified *L. plantarum* is foreign to that individual or is not present in the G.I. tract or mucosa of humans;

(b) the antigen is expressed intracellularly and/or on the cell surface; and/or

(c) the antigen is an immunogen.

25      22. A *Lactobacillus* bacterium which has been modified to express a heterologous antigen intracellularly and/or on the cell surface, to elicit an immune response to an individual and which can persist in the gastrointestinal tract of that individual for at least 7 days.

23. A *Lactobacillus* organism according to any of claims 18 to 22 which is *L. plantarum* or is for use in a vaccine.

24. An expression vector suitable for intracellular expression or exposure (on a cell surface) of a heterologous antigen, the expression vector being capable of

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providing expression in a *Lactobacillus plantarum* of the heterologous antigen under conditions existing in the gastrointestinal tract.

25. ~~A bacterium according to any of claims 19 to 24 for use in a method of prophylaxis or treatment of the human or animal body.~~

26. The use of a *Lactobacillus* bacterium which has been modified to express a heterologous antigen intracellularly and/or on the cell surface for the manufacture of a vaccine for an individual for whom the unmodified *L. plantarum* is foreign.

27. The use according to claim 26 wherein the unmodified *Lactobacillus* is *L. plantarum*, is not found in humans (the strain is endogenous) or is not present in the G.I. tract or mucosa of mammals.

28. ~~The use of a bacterium according to any of claims 19 to 24 in the manufacture of a vaccine.~~

29. The use according to claim 28 wherein the vaccine is adapted for oral administration and/or elicits an immune response on administration.

30. The use according to any of claims 26 to 29 for treating or preventing tetanus.